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LYSOGENIC PROPERTIES OF VARIANTS OF BACTERIA WITH MODIFIED CELL WALLS ON A MODEL OF SPHEROPLASTS AND L-FORMS

[Following is the translation of an article by N. S. Goryachkina and V. S. Levashev, II Moscow Medical Institute imeni N.I. Pirogova, published in the Russian-language periodical Vestnik AMN SSSR (Herald of the USSR Academy of Medical Sciences) Vol 20, 1965, pages 37-39. It was submitted on 2 Jun 1965. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The lysogenic condition of a bacterial cell, characterized by the potential capability of the prophage to convert into mature phage, may be viewed as an hereditary bacterial feature, and the preservation of the lysogenic condition in variants of bacteria with a modified cell wall, to which L-forms and spheroplasts may be regarded, is one of the supplementary indices of their genetic characteristics. Investigations by a number of authors (Mak Kvaylen; Borek and Ryan; Taubeneck and others) have demonstrated that the conversion of bacteria into spheroplasts and L-forms does not further the elimination of prophage in them. This is expressed in their capability to produce bacteriophage both spontaneously and under the influence of inducing factors.

The mission of the present investigation included the study of the preservation of the lysogenic condition in L-forms and spheroplasts of certain bacterial species, the study of their inductiveness under the influence of ultraviolet radiation, and also of the capability to produce bacteriophage in the various structural elements of L-forms, which was one of the means for studying genetic structure.

The objects of investigation were stable L-forms of lysogenic bacteria S. typhi K and S. typhimurium LT 2, and also glycine and penicillin spheroplasts, obtained by the generally accepted method from 9 cultures of S. typhimurium. It should be noted that the capability to produce phage was studied only in 18--24 hour spheroplasts, in the cultures of which bacillary forms were completely absent. The capability to produce bacteriophage was studied by the method of agar layers in the appropriate indicator cultures of S. typhi 34, S. typhimurium LT 2(1y) and 373. For studying the preservation of prophage in the various structural elements of L-forms, an L-culture of S. typhi L 152 was fractionated by the method of differential centriguging at 12,000--17,000 g, and also by filtration through the asbestos packing of a Sietz filter. Inductiveness of the variants was investigated by means of

irradiating them with ultraviolet rays in Petri dishes for 30--35 seconds in various media. These were physiological solution, Hanks solution, Hottinger broth (pH 7.4) and Hottinger broth with 20% sugar. In connection with the fact that the initial culture of S. typhi K formed phage in low titers ($4 - 10^2 - 2.2 \cdot 10^5$), the seeding of the material for the investigation which was obtained from that culture was carried out on agar layers without its preliminary dilution.

The investigations carried out made it possible to establish that the conversion of lysogenic bacteria S. typhimurium into glycine and penicillin spheroplasts does not contribute to the elimination of prophage. The spheroplasts of 9 cultures studied possessed the capability to produce phage spontaneously in the same titers as their initial bacterial cultures (table 1), however, the inductiveness of the spheroplasts was diverse.

Only one of the cultures obtained (No 465) possessed the capacity to be induced by ultraviolet rays following conversion into penicillin spheroplasts. The glycine spheroplasts of this same culture turned out to be non-inductive. The reason for the non-inductiveness of a large part of the spheroplasts studied is not clear: It is possible that suitable irradiation conditions were not selected. According to Borek and Ryan these have a definite importance when studying the inductiveness of spheroplasts. Data, obtained during the study of the inductiveness of penicillin spheroplasts from culture No 465, point to this same thought. In this study it was demonstrated that an increase in phage titer was observed only when the spheroplasts were irradiated in a broth of 20% sugar.

A study of stable L-forms of S. typhi K and S. typhimurium LT 2 made it possible to establish that L-forms, just like spheroplasts, have the capacity for the spontaneous production of bacteriophage, that is, the conversion of bacteria into stable L-forms does not eliminate their lysogenic condition. It can be seen from table 2 that L-forms produce phage in approximately the same titers as the initial cultures.

The study of the inductiveness of L-forms of S. typhi and S. typhimurium produced non-uniform results. Only the L-culture of S. typhi K turned out to be inductive. When it was irradiated with ultraviolet rays it was possible to note a small, but plain, increase in the number of negative colonies (table 3).

Irradiation of L-forms of S. typhimurium LT 2 with ultraviolet rays at various exposures in various media did not further an increase in the number of phage corpuscles, that is, in contrast to the initial culture the lysogenic system of the L-form of the stated species turned out to be non-inductive. The L-form of S. typhimurium LT 2, obtained under the influence of glycine, also turned out to be non-inductive. It was

not possible to establish the reason for the low level of inductiveness, in any case penicillin and glycine could not be the inhibitors of induction since the L-forms were washed thoroughly of the nutrient medium. The change of inductiveness was apparently connected with a change of microbial cells, since phage isolated here did not lose the capability to create lysogenic inducive systems.

The capability of the L-forms of S. typhi K to be induced by ultraviolet rays made it possible to study the presence of prophage in the various morphological elements of L-forms, fractionated with the help of differential centrifuging and filtration through bacterial filters. The results of these tests (V. S. Levashev) made it possible to establish that all the morphological elements of L-forms with a size greater than 0.3 microns are capable of producing bacteriophage after being irradiated with ultraviolet rays.

The results testified that the process of L-form formation does not contribute to the removal of prophage in the lysogenic cultures studied by us. The preservation of prophage in L-forms, established on the basis of spontaneous and inductive production of phage, indicates that the interrelationship between prophage and the chromosome in L-forms is not disrupted, and that the sectors of the chromosome where prophage is localized does not undergo noticeable changes during the process of L-form formation. The preservation of lysogenicity in L-forms is an additional criterion of their genetic nature, and it also creates the prerequisites for studying their intimate genetic structure.

Literature

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Table 1

Initial culture	Titer of spontaneous phage	Spheroplasts	Titer of spontaneous phage
LT 2	$5 \cdot 10^4$	Penicillin	$8 \cdot 10^4$
		Glycine	$7 \cdot 10^3$
No 465	$1.5 \cdot 10^4$	Penicillin	$2 \cdot 10^5$
		Glycine	$3 \cdot 10^4$
No 373/6X	$3 \cdot 10^4$	Penicillin	$3 \cdot 10^5$
		Glycine	$2 \cdot 10^5$

Table 2

S. typhi K		S. typhimurium LT 2	
Bacteria	L-forms	Bacteria	L-forms
$2.6 \cdot 10^2$	$1.6 \cdot 10^2$	$5 \cdot 10^4$	$3 \cdot 10^5$
$3.2 \cdot 10^2$	$8 \cdot 10^2$	$8 \cdot 10^4$	$3.5 \cdot 10^5$
$8 \cdot 10^1$	$1.6 \cdot 10^2$	$3 \cdot 10^4$	$6 \cdot 10^3$

Table 3

Number of test	Number of negative colonies in			
	S. typhi K		L-forms of S. typhi K	
	without induction	induction with ultraviolet rays	without induction	induction with ultraviolet rays
1	265	1 022	162	382
2	80	679	165	338
3	79	135	800	2 500